Z. Säugetierkunde 54 (1989) 141–152

ISSN 0044-3468

Chromosomal banding comparisons among American and European Red-backed mice, genus *Clethrionomys*

By W. S. Modi and Roswitha Gamperl

Biological Carcinogenesis Development Program, Program Resources, Inc., Frederick Cancer Research Facility, Frederick, USA and Institut für Medizinische Biologie und Humangenetik der Universität Graz, Austria

Receipt of Ms. 29. 10. 1987

Abstract

G-banded chromosomes are presented for five species of *Clethrionomys: C. glareolus, C. rufocanus, C. gapperi, C. californicus,* and *C. rutilus.* Similar data are available in the literature regarding *C. andersoni.* The presence of a shared derived autosomal reciprocal translocation allies *C. glareolus, C. gapperi, C. californicus* and *C. rutilus,* while the primitive condition is retained in *C. rufocanus* and *C. andersoni.* Intraspecific and interspecific variability in C-banding patterns is limited. Variation in Y chromosome size and/or morphology apparently occurs convergently in populations of three species. Interspecific chromosomal variation is much less in *Clethrionomys* than in other genera of arvicolid rodents, and *Clethrionomys* have speciated without concomitant structural chromosomal differences. Ribosomal DNA (rDNA) transcriptional activity was assayed among five different tissues from individuals belonging to three species using the silver staining procedure. Fibroblast cells had a significantly greater number of nucleolus organizer regions (Ag–NORs) than did femur bone marrow cells from the same specimen. Among similar tissues, intraspecific variability in the mean number of NORs per cell may be equal to or greater than interspecific differences. Sister chromatid exchange was measured in a lung fibroblast cell line of *C. rutilus* using 5-bromodeoxyuridine (BrdU) incorporation. The mean number of exchanges per cell (8.0) was found to be equal to or less than that reported in studies of other rodents. Meiotic chromosome analyses of *C. californicus* indicate that a sex vesicle is present during pachytene and that end-to-end association between the centromeric region of the acrocentric X and the small-sized, C-band negative Y occurs in diakinesis.

Introduction

The Holarctic rodent genus *Clethrionomys* (family Arvicolidae) is currently thought to contain seven (Honacki et al. 1982) or eight (Corbet 1978) species. These species are difficult to distinguish morphologically, and controversy has traditionally existed among classical taxonomists regarding the systematics of the group. Several of these species have fairly broad geographic distributions in either Eurasia (*C. glareolus* and *C. rufocanus*) or North America *(C. gapperi),* while one species *(C. rutilus)* exhibits a circumpolar distribution. The remaining forms are much more restricted in distribution either in the Palearctic *(C. andersoni, C. centralis, C. sikotanesis)* or in the Nearctic *(C. californicus).* Habitats occupied by these animals are principally mesic situations in coniferous, deciduous and mixed forests where an abundant litter is available, although the more specialized *C. rutilus* is found in a boreal, tundra habitat.

Previous comparative analyses have indicated that species in the family Arvicolidae exhibit some of the most extreme interspecific karyotypic variability yet observed among vertebrates (summarized by Modi 1987a). In this vein, the present study was undertaken because several earlier studies have suggested that species of *Clethrionomys* may be much more chromosomally conservative than are some closely related genera (Gamperl 1982a; Obara 1986). Results from the comparative analyses of G-banding and C-banding patterns from a total of six species (including information derived from the literature) are presented and compared with systematic arrangements of the genus that are based upon

other types of data. Additionally, results from silver-staining for the nucleolus organizer region, sister chromatid exchange and investigations on meiotic chromosomes are presented for several of these species.

Materials and methods

Karyotypic analyses were carried out on 17 specimens belonging to five species. Cells for chromosome preparations from *C. glareolus* and *C. rufocanus* were obtained from fibroblast cultures initiated from ear biopsies, grown in TC Medium Eagle, Earle BSS and supplemented with 20 % fetal calf serum. Cells from *C. rutilus* and all specimens of *C. gapperi* were obtained from fibroblast cultures initiated either from lung or ear biopsies and grown in McCoy's 5 A modified medium supplemented with 10 % fetal calf serum. Metaphase cells from three of the five specimens of *C. gapperi* were also derived from femur bone marrow following the yeast pretreatment procedure (Lee and Elder 1980), while preparations from *C. californicus* were obtained from spleen tissue (Modi 1985), femur bone marrow, or vertebral column bone marrow. Metaphase cell harvest, incubation, fixation, and slide preparation followed standard procedures. Slides were aged at 37 °C for 2–20 days before being banded.

G-bands were obtained on preparations from *C. glareolus* and *C. rufocanus* using a slightly modified version of the ASG technique (Sumner et al. 1971). Slides were incubated in 2× SSC (pH 7.0) at 60 °C for 8–16 h before being stained in 2 % Giemsa in sodium phosphate buffer (pH 6.8). G-bands from *C. gapperi, C. californicus* and *C. rutilus* were obtained by digestion with 0.025 % tgrypsin in Hanks' balanced salt solution for 20–200 sec, followed by dehydration in ethanol prior to staining in Giemsa (Seabright 1971). In Fig. 1 G-banded chromosomes are numbered following the designations proposed as phylogenetically primitive for the family Arvicolidae as defined by Modi (1987a). The chromosomes of *C. rufocanus* are arranged according to length, and the homologous elements from the other species paired accordingly (Fig. 1c).

C-bands were obtained from specimens of all five species following a variant of the BSG procedure of Sumner (1972). Slides were treated in 0.2 N HCl for 1 hr, followed by treatment in saturated $Ba(OH)_2$ for 2–20 min at 37 °C or 50 °C. Slides were then covered with 2× SSC and incubated at 60 °C for 1 hr prior to dehydration in ethanol and staining in 4 % Giemsa. Ag-NORs were obtained on the chromosomes of *C. gapperi, C. californicus* and *C. rutilus* following the AG-1 procedure of Bloom and Goodpasture (1976). Slides were flooded with a 50 % solution of $AgNO_3$ containing 0.03 % formalin and incubated at 60 °C for 1–4 hr. At least 50 silver-stained cells were examined per specimen and the number of chromosomes staining positively in each cell was recorded.

The frequency of sister chromatin exchange in a lung fibroblast cell line from *C. rutilus* was analyzed following a slight modification of the procedure of Perry and Wolff (1974). Cells were grown in the dark for 30 hr with 5-bromodeoxyuridine (BrdU) at a concentration of 30 µg/ml. Following slide preparation chromosomes were stained with Hoechst 33 258 (1 µg/ml) for 15 min, rinsed, dried and then flooded with 2× SSC and illuminated with long-wave UV light for 1 hr. Slides were stained in 2 % Giemsa in phosphate buffer (pH 6.8) for 2–4 min.

Finally, meiotic chromosomes were obtained from one male *C. californicus*. Seminiferous tubules were minced with curved scissors in a watch-glass in 2 ml of 0.7 % sodium citrate. An additional 8 ml of sodium citrate solution was added, and the mixture incubated at 37 °C for 25 min. Subsequent fixation and slide preparation followed traditional procedures.

The following specimens were examined: *C. rufocanus,* Sweden: Gällivare, 1 male. *C. glareolus,* Austria: Graz, 1 male, 1 female. Salzburg, 1 male. France: Savoie, 1 male, 2 females. *C. gapperi,* USA: Vermont, Chittenden Co., 1 male, 1 female. West Virginia, Randolph Co., 1 male; Virginia, Highland Co., 2 females. *C. californicus,* USA: Oregon, Tillamook Co., 1 male. Linn Co., 1 male, 2 females. *C. rutilus,* USA: Alaska, Fairbanks, 1 male.

Results

G-banded karyotypes were analyzed for all five species, including populations of *C. gapperi* from Vermont and Virginia and specimens of *C. glareolus* from two localities in Austria and one in France. No intraspecific differences in G-banding patterns were observed in either of these two species. Representative G-banded preparations for *C. rutilus* and *C. gapperi* are presented in Figures 1a–b. Illustrated in Figure 1c is a composite karyotype comparing the haploid complement from each of the five species. All species have $2n = 56$, $NF_a = 56$ with 25 pairs of acrocentric and one pair of small metacentric autosomes. Among the autosomes, pairs 1 and 9 of *C. rufocanus* differ from homologous

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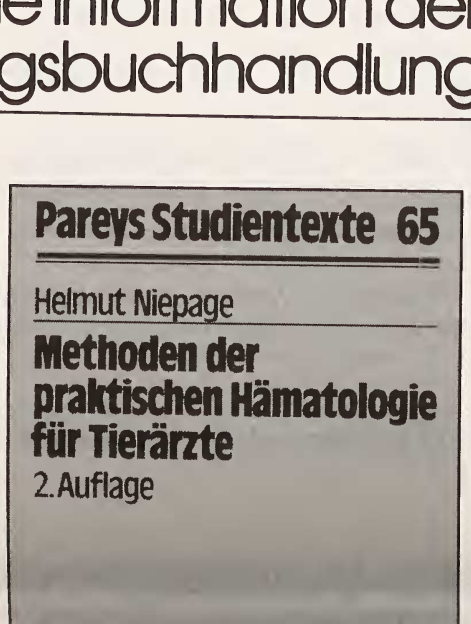

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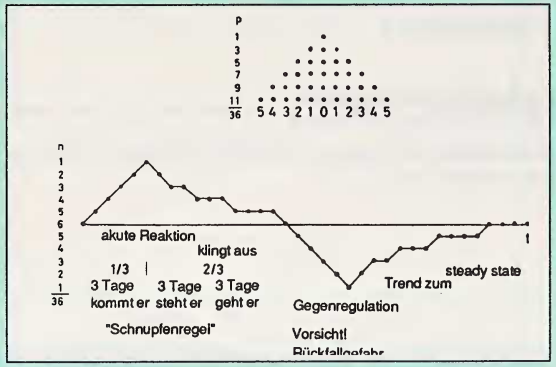

Abb. 18; Reaktionsverlauf (schematisch)

elements in the karyotypes of the other four species by a reciprocal translocation. Complete homology appears to exist among the remaining autosomes and the X chromosome in all species, however, the variable quality of banding of several smaller-sized pairs from *C. rufocanus* (nos. 17, 22, and 25) makes these comparisons equivocal. Intraspecific and interspecific variability exists in the size and morphology of the Y chromosome. The Y in *C. rufocanus* and *C. gapperi* is a medium sized acrocentric. In *C. glareolus* from Austria and in *C. rutilus* it is metacentric, while in the *C. glareolus* specimen from France the element is submetacentric. The Y in *C. californicus* is an extremely small-sized acrocentric.

C-bands were examined from all five species, including specimens of *C. glareolus* from Austria and France, and *C. gapperi* from Vermont, Virginia and West Virginia. Presented in Figures 2a–b are C-banded karyotypes of *C. gapperi* and of *C. rutilus*, while C-banded karyotypes of *C. rufocanus, C. glareolus* and *C. californicus* are found elsewhere (Gamperl 1982a; Modi 1987b). Among all species, autosomal C-bands are found predominantly centromerically. The sizes of centromeric autosomal C-bands in *C. rufocanus* and *C. glareolus* (Austria) are larger than those in the other species or in the French specimen of *C. glareolus*. Further, both members of pair 27 in *C. rufocanus* are almost completely C-band positive. In the specimen of *C. gapperi* from West Virginia two different pairs of autosomes exhibit C-band heteromorphisms. In the first pair, one element has a large-sized centromeric C-band and C-band positive short arms, both of which are absent in the homologue. In the second pair, one element is completely heterochromatic (Fig. 2a). The other specimens of *C. gapperi* did not show this intraindividual C-band variation. The Y is completely C-band positive in all species except *C. californicus*, where it is C-band negative.

Metaphase cells from a total of five different tissues (femur bone marrow, vertebral column bone marrow, spleen, ear fibroblasts, and lung fibroblasts) from six specimens belonging to three species (*C. gapperi, C. californicus,* and *C. rutilus*) were examined using silver staining (s. Table). Representative cells from *C. gapperi* and *C. californicus* are shown in Figs. 2c–d. All Ag-NORs examined were found pericentromerically and never interstitially or telomerically. The mean number of Ag-NORs per cell ranged from 2.46 to 3.25 for femur bone marrow cells among the four specimens examined, and from 5.58 to 8.37 for fibroblast cells among the three specimens examined. The modal number of Ag-NORs per cell was lower for femur bone marrow cells than for cells from the other tissue types. The range between the minimum and maximum number of NORs staining per cell showed little variability among all specimens and tissues examined (s. Table).

Differences among the mean number of NORs staining per cell (s. Table) were tested for statistical significance using a one-way analysis of variance (ANOVA) in each of the following five comparisons: 1. *C. gapperi* 6 femur bone marrow versus ear fibroblast cells ($F = 106.9$, $p < 0.001$), 2. *C. gapperi* 7 femur bone marrow versus ear fibroblast cells ($F = 257.5$, $p < 0.001$), 3. *C. californicus* 3 femur bone marrow versus spleen cells ($F = 0.53$, $p > 0.10$), 4. femur bone marrow cells from *C. gapperi* 6, *C. gapperi* 7, *C. californicus* 2 and *C. californicus* 3 ($F = 3.34$, $0.02 < p < 0.05$) and 5. fibroblast cell lines from *C. gapperi* 6, *C. gapperi* 7 and *C. rutilus* 1 ($F = 78.79$, $p < 0.001$). These results indicate significant differences among group means in all analyses except the comparison of *C. californicus* 3 femur bone marrow versus spleen cells.

Next, two of the above analyses were repeated after deleting one sample from each. Three of the four femur samples (all except *C. californicus* 2) were compared and a lack of significance was found ($p > 0.10$). Similarly, two of the three fibroblast samples (except *C. gapperi* 6) were compared and the ANOVA was non-significant ($p > 0.10$). These last two analyses indicate that the two deleted samples differ significantly from the remaining samples in their respective groups which collectively represent rather homogeneous populations. The frequency distributions of the samples analyzed by the ANOVAs are plotted in Fig. 4.

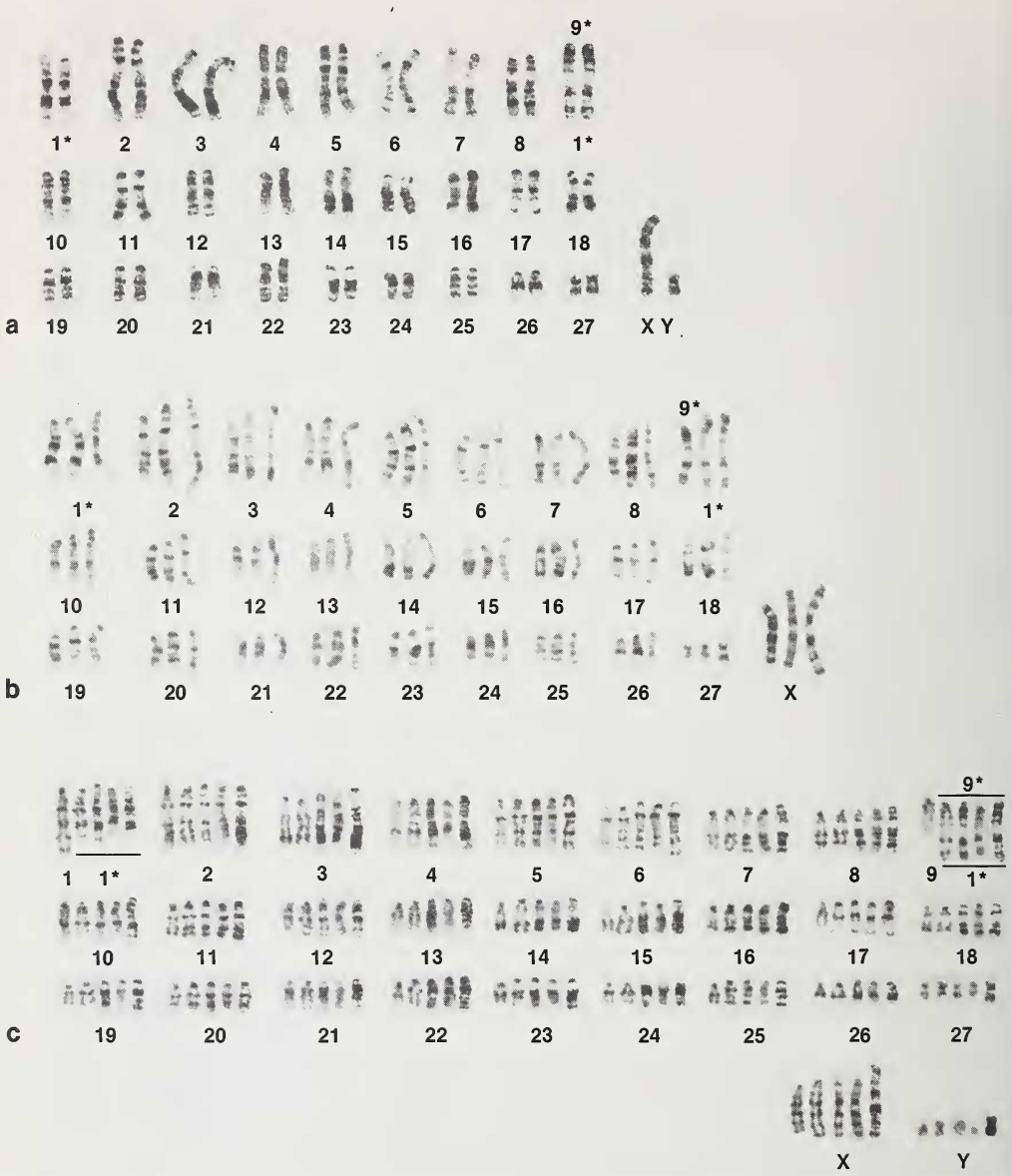

Fig. 1. G-banded karyotypes of *Clethrionomys*. a: Male *C. rutilus;* b: Haploid complements of a female *C. gapperi* from Vermont at three different stages of condensation; c: Composite karyotype comparing the haploid complement from each of five species. Elements, arranged from left to right in each set, are from *C. rufocanus, C. glareolus, C. gapperi, C. californicus,* and *C. rutilus*. Asterisks indicate chromosomes that have undergone rearrangement from the primitive condition as proposed by Modi (1987a)

Table. **Results from the silver staining analyses for the nucleolus oganizer region**
Standard statistics regarding the number of Ag-NORS per cell among different tissues are given for three species of *Clethrionomys*

Specimen	Tissue	No. Cells	Mean	SD	Mode	Range
C. gapperi 6	f[1]	241	2.80	2.58	0	0–12
C. gapperi 6	e	169	5.58	2.78	6	0–11
C. gapperi 7	f	298	3.24	2.88	0	0–12
C. gapperi 7	e	117	8.37	3.05	10	0–13
C. californicus 2	f	141	2.46	2.07	2	0–9
C. californicus 3	f	115	3.25	3.07	0	0–11
C. californicus 3	s	105	3.54	2.80	3	0–12
C. californicus 4	v	123	5.16	3.22	3	0–13
C. rutilus 1	l	51	8.28	2.52	10	0–13

[1] Tissue sources from which metaphase chromosomes were derived: f = femur bone marrow, e = ear fibroblast cell line, s = spleen, v = vertebral column bone marrow, l = lung fibroblast cell line

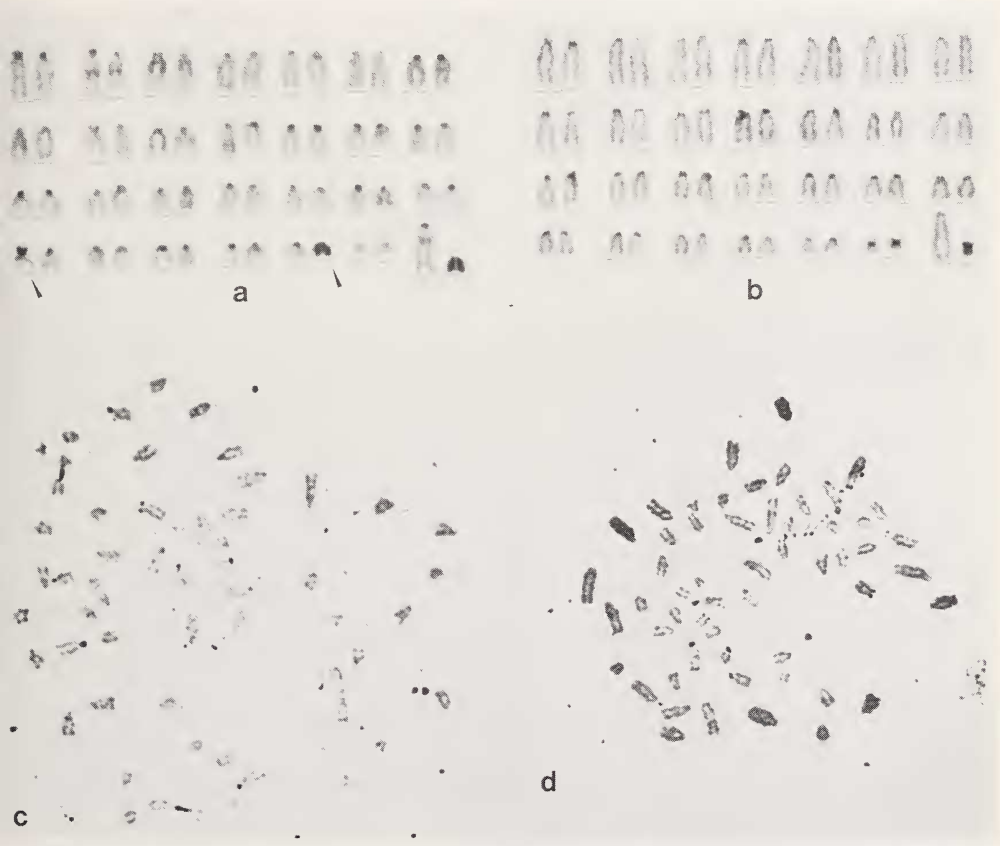

Fig. 2. a: C-banded karyotype of a male *C. gapperi* from West Virginia with the arrowheads illustrating autosomal C-band heteromorphisms; b: C-banded karyotype of a male *C. rutilus*; c: Silver stained metaphase cell of female *C. gapperi* 7 from Vermont derived from an ear fibroblast cell line; d: Silver stained metaphase cell from female *C. californicus* 4 derived from vertebral bone marrow. In both (c–d) Ag-NORs are visible at the centromeres of eleven acrocentric chromosomes

The frequency of sister chromatid exchange was recorded from 16 cells in a male specimen of *C. rutilus*. A representative metaphase cell is shown in Figure 3a. The mean number of exchanges per cell plus or minus one standard deviation was 8.0 ± 3.3 with a range of 5–14.

A total of 21 pachytene and 11 diakinesis cells was observed in meiotic preparations from a single male *C. californicus*. Twenty-seven autosomal bivalents and a conspicuous sex vesicle were observed in the pachytene cells (Fig. 3b). At diakinesis 27 autosomal bivalents were apparent, and an end-to-end association existed between the X and Y (Fig. 3c). It appears as though the centromeric end of the X synapses with the Y in the Giemsa stained cells.

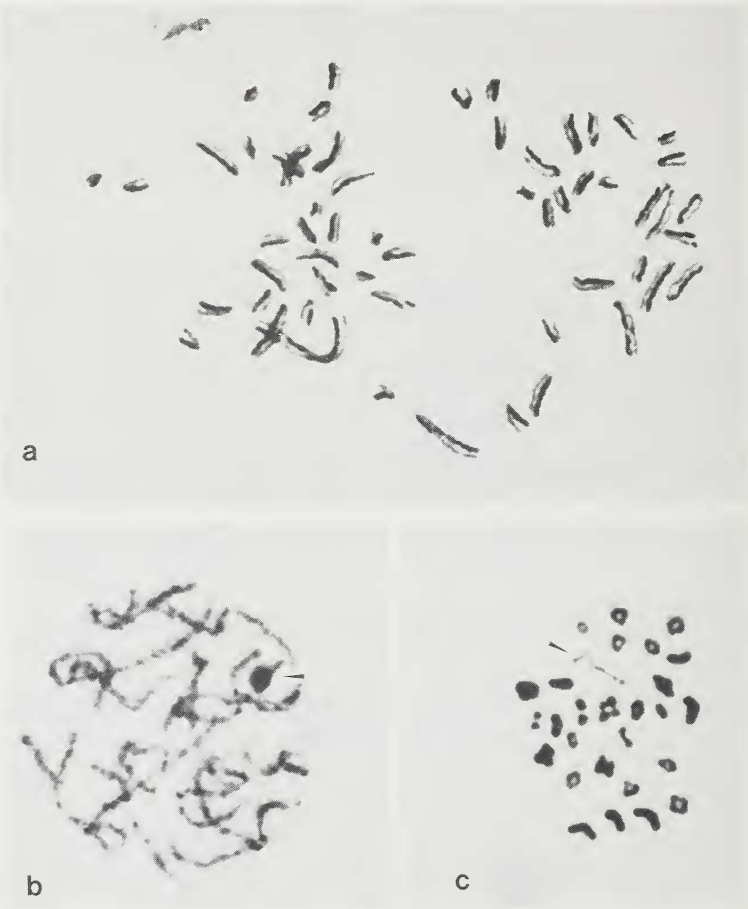

Fig. 3. a: Metaphase cell of *C. rutilus* after BrdU incorporation and modified FPG staining. An unusually large number of sister chromatid exchanges (eleven) may be noted. b: Giemsa stained pachytene cell from a male *C. californicus*, with the arrowhead pointing out the sex vesicle; c: Giemsa stained diakinesis cell from a male *C. californicus* with the arrowhead indicating the free end of the X